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Genomic Imprinting in Turner Syndrome: Effects on Response to Growth Hormone and on Risk of Sensorineural Hearing Loss

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Context: Evidence exists for X-linked parent-of-origin effects in Turner syndrome, because phenotypic and cognitive profiles differ between 45,$X^{maternal}$ and 45,$X^{paternal}$ individuals.

Objective and Design: We evaluated the parent-of-origin effect of the intact X chromosome on spontaneous growth, GH-stimulated height gain, and frequency of sensorineural hearing loss in 54 subjects with Turner syndrome recruited from a Canadian randomized, controlled trial of GH supplementation to adult height.

Methods and Results: Microsatellite analyses revealed that 72% of nonmosaic 45,X subjects retained an $X^{maternal}$, whereas 86% of nonmosaic 46,X,i(Xq) subjects carried an intact $X^{paternal}$. No significant differences were noted between $X^{maternal}$ and $X^{paternal}$ subjects for parents' heights, birth weight and length, and height, age, or bone age at study entry. In all subjects, and in those with $X^{maternal}$, baseline height SD score correlated with midparental height (all: $r = 0.511$, $P < 0.001$; $X^{maternal}$: $r = 0.535$, $P = 0.001$) and with mother's height (all: $r = 0.510$, $P < 0.001$; $X^{maternal}$: $r = 0.574$, $P < 0.001$) but only weakly with father's height (all: $r = 0.334$, $P = 0.015$; $X^{maternal}$: $r = 0.292$, $P = 0.094$). Using a linear model including age and height at GH initiation, subjects with $X^{maternal}$ had a greater mean height gain than those with $X^{paternal}$ (SD score difference and 95% confidence interval for all karyotypes was +0.43 and 0.04–0.82, $P = 0.030$, and for 45,X was +0.64 and 0.06–1.21, $P = 0.031$); X-linked imprinting explained 36–53% of the GH response. After pure tone audiometry testing, $X^{maternal}$ subjects were also less likely ($P = 0.040$) to have sensorineural hearing loss than $X^{paternal}$ subjects.

Conclusion: This study provides evidence of an X-linked imprinting effect on GH response and on sensorineural hearing loss in Turner syndrome and should fuel the search for candidate genes. (***J Clin Endocrinol Metab* 91: 3002–3010, 2006**)

TURNER SYNDROME (TS) is the most prevalent female sex chromosomal disorder, affecting one in 1800–2500 live-born girls (1–3). TS results from complete or partial monosomy of the X chromosome; this may exist in nonmosaic or mosaic forms, with or without the presence of a normal 46,XX or, occasionally, 46,XY cell line. Ninety-nine percent of fetuses with TS do not reach term; the 1% that survive exhibit, to different degrees, a wide spectrum of characteristic physical and neuropsychological features (4), including short stature, ovarian dysgenesis (leading to sexual infantilism and infertility), lymphedema, cardiovascular defects, renal malformations, and hearing loss. Individuals with TS may also exhibit social and behavioral problems as well as cognitive deficits affecting nonverbal learning abilities and visuospatial skills (3).

The short stature in TS is characterized by growth retardation that begins in intrauterine life, persists throughout childhood, and worsens during puberty because of the absence of the pubertal growth spurt. The mean adult height of untreated women with TS is approximately 20 cm below that of the general female population from the same ethnic origin. The growth failure is not because of deficiency of GH secretion but in part because of haploinsufficiency of the pseudoautosomal gene *SHOX* (**s**hort stature homeobox-containing gene; Xp22.33 and Yp11.32) (5). The encoded transcription factor plays a role in growth plate morphology (6) and in the regulation of the cell cycle and apoptosis in chondrocytes (7).

Many studies have demonstrated significant increases in height velocity (8–10) in response to GH treatment (GH-Tx), and recombinant GH-Tx is now approved for use in patients with TS in many countries. We recently published the first randomized, controlled trial of GH-Tx to adult height in TS (mean age, 21 yr) that established that GH also increases adult height in TS; the mean height difference between the GH-treated and the control groups was 7.3 cm [95% confidence interval (CI), 5.4–9.2 cm] (11).

The response to GH in patients with TS varies widely (11). Many factors may explain this variability, including age, bone age, and height at initiation of GH-Tx and timing of estrogen replacement therapy (11, 12). However, no data exist on the possible contribution of the parental origin of the intact X chromosome (X^{intact}), in other words, a possible

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Abbreviations: CI, Confidence interval; GH-Tx, GH treatment; SDS, SD score; SNHL, sensorineural hearing loss; TS, Turner syndrome; X^{intact}, intact X chromosome; X^{mat}, maternal X^{intact}; X^{pat}, paternal X^{intact}.

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genomic imprinting effect on treatment response. Genomic imprinting is an epigenetic phenomenon referring to the differential expression of genes depending on their parent of origin and is believed to have evolved in mammals to regulate, in part, the dosage of developmentally sensitive genes (13). In humans, dysregulation of imprinting mechanisms has been linked to altered viability, fetal and postnatal growth, neurological development, and behavior (14, 15).

TS provides a valuable clinical model to investigate the impact of putative X-linked imprinted genes on growth and neurocognitive development, because the X^{intact} can be of either maternal (X^{mat}) or paternal (X^{pat}) origin. Evidence for imprinting of some human X-linked genes is accumulating (16–21). For example, girls with TS who retain an X^{mat} may be at increased risk for cardiovascular anomalies, neck webbing (16), and poorer social cognition (17). An effect of imprinting on growth in TS has also been suggested, because the pretreatment height of girls retaining an X^{mat} correlates with maternal but not paternal height (16).

To look for imprinting effects in TS, we investigated the role of the parental origin of the X^{intact} on growth, including birth weight, birth length, and height at study entry, and on height gain in response to GH-Tx using a subset of subjects from the Canadian TS study (11). Because sensorineural hearing loss (SNHL) inflicts significant morbidity on affected individuals, we also investigated the relationship of SNHL to parental origin of the X. As has been reported previously (22, 23), the majority of subjects in our study inherited their X^{intact} from their mother. These subjects had greater mean GH-stimulated height gain and were less likely to have SNHL than those with X^{pat}. This study provides evidence of an imprinting effect on GH response and on SNHL in TS.

Subjects and Methods

Subjects

The subjects eligible for this study comprised a subset of 114 of the 154 girls with TS previously enrolled in a Canadian randomized, controlled trial of GH-Tx to adult height. At entry into the core study, subjects were randomized to either a GH-Tx group (0.30 mg/kg·wk Humatrope; Eli Lilly Canada Inc., Toronto, Ontario, Canada) or a nontreated control group. Pubertal induction was standardized with sex steroids (ethinyl estradiol and medroxyprogesterone acetate) for both GH-Tx and control subjects. Participants were followed to near-adult height, defined by an annual height velocity of less than 2.0 cm/yr and bone age of at least 14 yr. Details of the primary study design and results are described elsewhere (11). Subjects were considered eligible to participate in this genetic extension study if they met the following inclusion criteria: 1) peripheral blood karyotype consisted of 45,X; 46,X,del(Xp); 46,X,i(Xq); or 45,X mosaicism with no 46,XX normal cell line; and 2) willingness and availability of biological mother to provide a peripheral blood sample (paternal blood sampling was excluded to avoid the potential ethical problem and experimental bias of nonpaternity). Subjects with any chronic illness likely to have an impact on growth and subjects taking any medications known to affect growth were excluded, as were those with a karyotype that included Y chromosome material.

After signed informed consent, 56 subjects (GH-Tx n = 36; control n = 20; Caucasian n = 47; Asian n = 4; Hispanic n = 2; mixed parentage including Caucasian n = 3) were enrolled in the genetic study. The follow-up visit for participation in this study was scheduled to occur at least 1 yr after the end of the core study. At the time of this visit, subjects were remeasured to determine whether additional growth had occurred. Subjects also underwent an audiology examination to determine tympanic membrane function by impedance tympanometry and hearing threshold at various sound frequencies by standard audiometry. Subjects with any abnormality on standard audiometry also underwent otoacoustic emissions testing to look for additional evidence of a sensorineural component to their hearing loss.

To address the possibility of selection bias, baseline characteristics of participating subjects were compared with those of nonparticipating eligible subjects (Fig. 1 and Table 1).

Genotyping of microsatellites

Duplicate peripheral blood samples were drawn from the subjects and their mothers, and leukocyte DNA was extracted as previously described (24). PCR conditions were optimized for 14 highly polymorphic X chromosome microsatellites (DXS7100, DXS1053, CYBB, DXS538, DXS1068, DXS1003, DXS1204, AR, DXS981, DXS1125, DXS986, DXS1120, DXS1047, and DXS102) chosen after their high degree of heterozygosity (mean = 78%) and their allele frequencies (≤47%). Most microsatellites were amplified with commercially available primers (MapPairs Human Markers) through Invitrogen Corp. (Burlington, Ontario, Canada) with the exception of the AR polymorphism for which the forward primer 1, 5′-TCCAGAATCTGTTCCAGAGCGTGC-3′, and the reverse primer 3, 5′-CTCTACGATGGGCTTGGGGAGAAC-3′, were used as described (25). Specifications regarding allele number and size were obtained through the Genome DataBase web site (http://www.gdb.org). Details on microsatellite-specific PCR may be found online as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org.

Parental origin assignment

To determine the parental origin of the X^{intact}, genotype comparisons between mothers and their daughters were conducted for different combinations of microsatellites depending on the daughter's karyotype. In the case of a non-45,X subject, only markers located on the hemizygous portion of the X chromosome were studied. For each microsatellite, the size of the allele on the X^{intact} chromosome was first determined using the M13mp18 plasmid sequence generated as indicated in the Sequenase version 2.0 DNA Sequencing Kit protocol (USB, Amersham Biosciences Corp., Baie d'Urfé, Québec, Canada). Only alleles showing rare frequency (≤0.15 in the case of a maternal allele assignment) were retained with the aim of calculating a discrimination power (allele $\text{frequency}_1 \times$ allele $\text{frequency}_2 \times$ allele frequency_n). The discrimination power allows estimation of the probability of false assignment of parental origin. Because no paternal blood was available, we required a discrimination power of less than 0.001 to assign maternal origin to the X^{intact} (mean of nine microsatellites) and less than 0.01 in the case of an intact X^{pat} chromosome (mean of seven microsatellites).

Statistical analysis

The differences in X^{mat} and X^{pat} distributions between 45,X, 45,X/mosaic, and 46,X,i(Xq) groups were assessed using Fisher's exact test. Age-specific and adult height SD scores (SDS) were determined using the Lyon *et al.* (26) growth standards for patients with TS. Although the Lyon growth curve is based on cross-sectional data, it has been validated in the present population (11), and on average, untreated subjects followed curves of constant height SDS over time. This is not true for patients with TS if followed on the National Center for Health Statistics growth curves, making the latter inappropriate for growth analyses in TS. The influence of parental origin of the X^{intact} on baseline height SDS was evaluated, within each parental origin group, by a linear regression of pretreatment height SDS separately upon mother's height, upon adjusted father's height, and upon adjusted midparental height (27). Pearson correlations are reported for these regressions. To estimate the contribution of a parental origin effect on the response to GH-Tx, we examined a linear model of change in height SDS from baseline to last available measurement for GH-Tx subjects, using explanatory variables of age and height SDS at initiation of GH-Tx and parental origin of the X^{intact}. We calculated the percentage of the total height gain attributable to imprinting by dividing this figure by 1.2 SDS, the total height gain achieved in the Canadian randomized, controlled trial of GH-Tx to adult height (11). Finally, Fisher's exact test was used to examine the influence of parental origin of the X^{intact} on presence or absence of sensorineural hearing deficit.

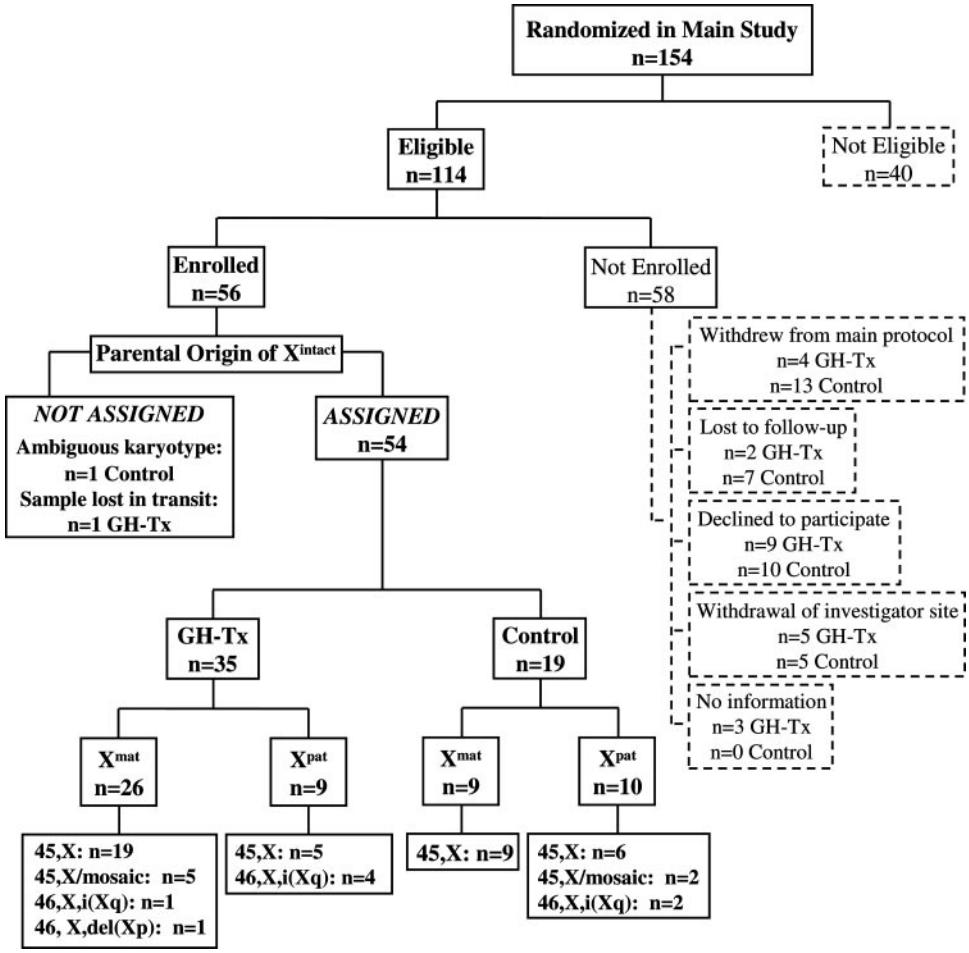

FIG. 1. Outline of study participation and nonparticipation.

Results

Assignment of the parental origin of the X^{intact}

Parental origin of the X^{intact} was assigned in 54 of the 56 subjects (X^{mat} = 35; X^{pat} = 19). One case of previously unsuspected 46,XX mosaicism was detected; this subject was excluded from the study because microsatellite analysis gave biallelic patterns in duplicate blood samples. One subject's samples were lost during shipment. Karyotypes of the 54 analyzable subjects were 45,X (n = 39); 46,X,i(Xq) (n = 7); 45,X/46,X,i(Xq) (n = 4); 45,X/46,X,del(Xq) (n = 1); 46,X,del(Xp) (n = 1); 45,X/46,X,del(Xp) (n = 1); and 45,X/46,X,der(X) nuc ish Xcen (DXZ1x2) (n = 1). Details of study participation and nonparticipation are presented in Fig. 1.

Distribution of X^{mat} and X^{pat} subjects by karyotype

Distribution of the X^{mat} and X^{pat} among subjects with a 45,X karyotype was consistent with published findings (22, 23), because 72% (n = 28) of the 45,X subjects retained an X^{mat} and 28% (n = 11) retained an X^{pat}. Similarly, among the 45,X/mosaic subjects, 71% (n = 5) had an X^{mat} and 29% (n = 2) had an X^{pat} in the 45,X cell line.

Isochromosomes of the long arm of the X chromosome [i(Xq)] are the most frequent X-chromosomal structural abnormality in TS and the second most common karyotype (28–30). In previous studies of the parental origin of the X^{intact}, mosaic (in combination with 45,X cell lines) and nonmosaic forms of i(Xq) have been analyzed as one category, with i(Xq) equally likely to be maternally or paternally derived (31). Because this X^{mat}:X^{pat} ratio (1:1) deviates from the 2.5:1 ratio seen in our subjects with 45,X karyotype, this suggested to us that nonmosaic 46,X,i(Xq) karyotypes should be analyzed separately. Six (86%) of the seven subjects with a nonmosaic 46,X,i(Xq) karyotype retained an intact X^{pat}. This distribution was significantly different from the other karyotype groups [46,X,i(Xq) *vs.* 45,X and 45,X/mosaic groups combined: $P = 0.006$; 46,X,i(Xq) *vs.* 45,X alone: $P = 0.007$].

Effect of parental origin of the X^{intact} on auxological parameters

Table 2 provides comparative data for auxological and other parameters at baseline and after GH-Tx, grouped according to origin of the X^{intact}. There were no statistically significant differences between X^{mat} and X^{pat} groups at baseline, either overall or by treatment group. There was no evidence for a parent-of-origin effect on birth weight or length, and the X^{mat} and X^{pat} groups were comparable in terms of maternal and paternal heights, suggesting that their genetic target heights should, theoretically, be similar. Re-

TABLE 1. Auxology of participating *vs.* nonparticipating subjects at baseline

Characteristics	Participants			Nonparticipants			P^a		
	All mean ± SD (n)	GH-Tx mean ± SD (n)	Control mean ± SD (n)	All mean ± SD (n)	GH-Tx mean ± SD (n)	Control mean ± SD (n)	All	GH-Tx[b]	Control[b]
Age (yr)	10.0 ± 1.7 (54)	9.8 ± 1.7 (35)	10.4 ± 1.6 (19)	10.7 ± 1.7 (60)	10.8 ± 1.7 (24)	10.6 ± 1.7 (36)	0.037[c]	0.031[c]	0.626
Bone age (yr)	8.7 ± 1.4 (53)			8.7 ± 1.4 (49)			0.963		
Height (cm)	119.0 ± 8.1 (54)			121.0 ± 7.7 (60)			0.183		
Height SDS (Lyon)	0.0 ± 0.9 (54)	0.1 ± 0.9 (35)	−0.3 ± 0.8 (19)	−0.1 ± 0.9 (60)	−0.3 ± 0.9 (24)	0.0 ± 0.9 (36)	0.654	0.107	0.213
Birth weight (kg)	2.8 ± 0.9 (49)			2.0 (1)					
Birth length (cm)	46.5 ± 4.5 (28)			NA (0)					
Mother's height (cm)	160.2 ± 7.9 (53)			160.2 ± 6.6 (53)			0.996		
Father's height (cm)	173.5 ± 8.6 (52)			173.2 ± 7.2 (52)			0.817		
Midparental height (sex adjusted) (cm)	160.4 ± 6.8 (52)			160.2 ± 5.8 (52)			0.886		

Participants refers to enrolled patients for whom parental origin was assigned. Nonparticipants refers both to patients who were enrolled but for whom parental origin could not be assigned (n = 2) and to those who were not enrolled in the study (n = 58). Midparental heights were calculated from the mean of mother's height and adjusted father's height; fathers' heights were sex adjusted by subtracting 13 cm (to account for the mean difference between adult male and adult female heights) (27). NA, Not available.

[a] *P* values for comparisons between participants and nonparticipants.

[b] *P* values for comparisons between participants and nonparticipants within treatment categories. Height variables were examined within a model including explanatory variables of parental X chromosome origin, baseline height SDS, and age at initiation of treatment.

[c] Significant at $P < 0.05$.

stricting the comparison of parental origin groups to subjects with a nonmosaic 45,X karyotype gave similar results.

Contribution of parental height variables to subjects' baseline height SDS

Midparental height influences the height of untreated subjects with TS (27), likely reflecting the effect of autosomal stature-determining genes. In our subjects, when all karyotype groups were combined, baseline height SDS was highly correlated with sex-adjusted midparental height (r = 0.511; $P < 0.001$), with mother's height (r = 0.510; $P < 0.001$), and less strongly with father's height (r = 0.334; $P = 0.015$). A putative contribution of maternal X height-determining genes was supported by the strong correlation in X^{mat} subjects between baseline height SDS and maternal height (r = 0.574; $P < 0.001$), which was not seen with paternal height (r = 0.292; $P = 0.094$; Fig. 2, A and B). This effect was not seen in X^{pat} subjects, whose baseline height SDS showed a weaker correlation with maternal height (r = 0.476; $P = 0.046$) and paternal height (r = 0.403; $P = 0.097$; Fig. 2, C and D). This suggests that the correlation between the subjects' baseline height SDS and their midparental height observed overall (r = 0.511; $P < 0.001$) or within X^{mat} (r = 0.535; $P = 0.001$) or X^{pat} (r = 0.503; $P = 0.033$) groups may be attributable primarily to height genes on the X^{mat} chromosome in X^{mat} subjects and to autosomal genes in X^{pat} subjects. However, these results cannot completely exclude the contribution of height genes on autosomes in X^{mat} subjects or on X^{pat} chro-

TABLE 2. Auxology of participating subjects at baseline and at most recent height

Characteristics	X^{mat}			X^{pat}			P^a		
	All mean ± SD (n)	GH-Tx mean ± SD (n)	Control mean ± SD (n)	All mean ± SD (n)	GH-Tx mean ± SD (n)	Control mean ± SD (n)	All	GH-Tx[b]	Control[b]
Baseline									
Age (yr)	10.0 ± 1.6 (35)	9.9 ± 1.6 (26)	10.2 ± 1.8 (9)	10.0 ± 1.7 (19)	9.5 ± 1.8 (9)	10.5 ± 1.5 (10)	0.942	0.521	0.683
Bone age (yr)	8.8 ± 1.4 (35)			8.6 ± 1.2 (18)			0.601		
Height (cm)	119.8 ± 8.5 (35)			117.5 ± 7.3 (19)			0.329		
Height SDS (Lyon)	0.1 ± 0.9 (35)	0.2 ± 1.0 (26)	0.0 ± 0.6 (9)	−0.3 ± 0.8 (19)	0.0 ± 0.6 (9)	−0.6 ± 0.9 (10)	0.093	0.622	0.141
Birth weight (kg)	2.9 ± 1.0 (32)			2.8 ± 0.6 (17)			0.756		
Birth length (cm)	46.2 ± 4.7 (20)			47.1 ± 4.2 (8)			0.625		
Mother's height (cm)	159.7 ± 8.9 (35)			161.1 ± 5.6 (18)			0.565		
Father's height (cm)	174.1 ± 9.3 (34)			172.4 ± 7.3 (18)			0.502		
Midparental height (sex adjusted) (cm)	160.5 ± 7.4 (34)			160.2 ± 5.6 (18)			0.906		
At most recent height									
Age (yr)		20.4 ± 2.4 (26)	20.7 ± 1.8 (9)		20.7 ± 2.9 (9)	21.2 ± 2.8 (10)		0.744	0.642
Years on main study		5.9 ± 1.8 (26)	5.2 ± 1.7 (9)		5.3 ± 3.0 (9)	4.3 ± 2.5 (10)		0.453	0.360
Height SDS (Lyon)		1.2 ± 0.8 (26)	0.0 ± 1.0 (9)		0.8 ± 0.7 (9)	−0.1 ± 1.1 (10)		0.030[c]	0.348
Change in height SDS (Lyon)		1.1 ± 0.6 (26)	0.1 ± 0.8 (9)		0.8 ± 0.7 (9)	0.5 ± 0.7 (10)		0.030[c]	0.348

Midparental heights were calculated from the mean of mother's height and adjusted father's height; fathers' heights were sex adjusted by subtracting 13 cm (to account for the mean difference between adult male and adult female heights) (27).

[a] *P* values for comparisons between parental X chromosome origin groups.

[b] *P* values for comparisons between parental X chromosome origin groups within treatment categories. Height variables were examined within a model including explanatory variables of parental X chromosome origin, baseline height SDS, and age at initiation of treatment.

[c] Significant at $P < 0.05$.

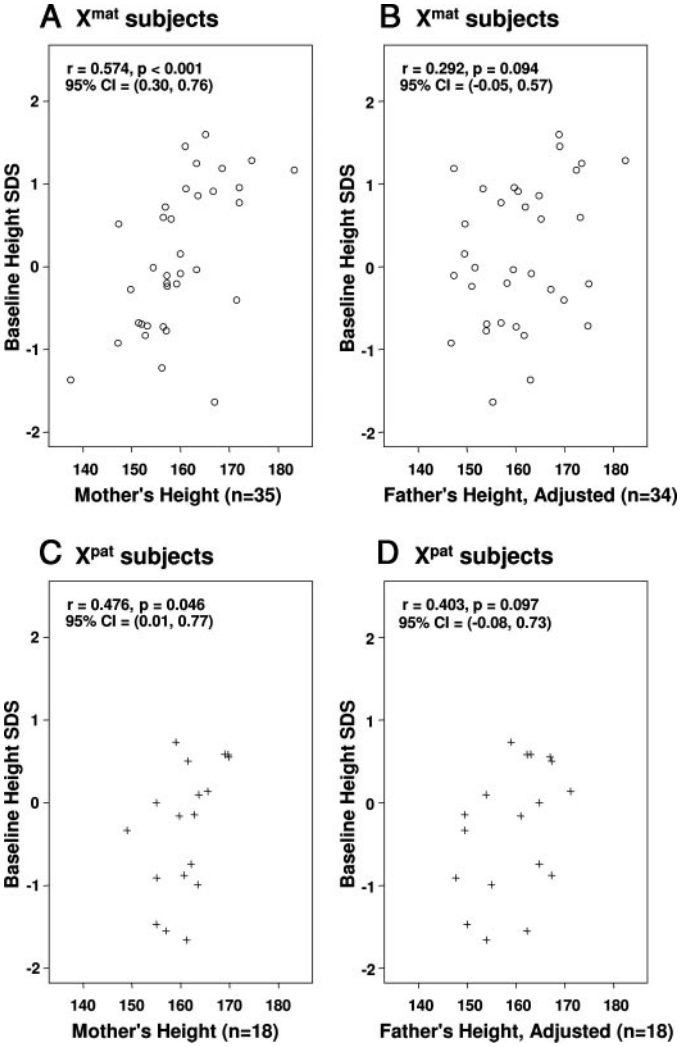

FIG. 2. Baseline height SDS of subjects with TS in relation to parental height. In X^{mat} subjects, a significant correlation was seen between baseline height SDS and mother's height (A), whereas no significant correlation was found with father's height (B). In X^{pat} subjects, a weak significant correlation was seen between baseline height SDS and mother's height (C), whereas no significant correlation was found with father's height (D). Adjusted refers to the sex-adjusted correction of father's height by the subtraction of 13 cm (the difference between male and female mean adult height).

mosome in the case of X^{pat} subjects. Similar results were found even when this analysis was restricted to the nonmosaic 45,X karyotype group only.

Influence of the parental origin of the X^{intact} on response to GH-Tx

At the most recent post-study height measurement, neither the mean age nor the number of years in the primary study differed between the GH-Tx and control groups or between the X^{mat} and X^{pat} groups (Table 2). X^{mat} and X^{pat} control subjects had comparable mean adult height SDS. In contrast, mean adult height SDS of GH-Tx subjects differed significantly between X^{mat} and X^{pat} subjects (1.2 ± 0.8 SDS *vs.* 0.8 ± 0.7 SDS, respectively; $P = 0.030$), as did the change in height SDS from baseline (1.1 ± 0.6 *vs.* 0.8 ± 0.7 SDS; $P = 0.030$). The difference in adult height SDS between GH-treated groups of differing parental X chromosome origin is illustrated in Fig. 3.

Growth data from this and other TS studies (11, 32) consistently demonstrate that age and height SDS at GH initiation are important explanatory variables with respect to total height gain and thus should be included in regression models of GH response. In the present data (all karyotype groups combined), both age ($P = 0.001$) and height SDS ($P = 0.005$) at initiation of GH-Tx were significant explanatory variables when incorporated into a linear model to examine the effects of parental origin of the X^{intact} on change in height SDS for GH-Tx subjects. X^{mat} subjects had a greater mean response to GH-Tx than X^{pat} subjects of 0.43 SDS ($P = 0.030$; 95% CI, 0.04–0.82); this imprinting effect explained 36% of the total height gain with GH-Tx. Analyzing height in centimeters, X^{mat} subjects had a greater mean response to GH-Tx than X^{pat} subjects of 3.37 cm ($P = 0.017$; 95% CI, 0.66–6.09).

Limiting these analyses to the 45,X subjects, the same explanatory variables of age ($P = 0.004$) and height SDS ($P = 0.037$) at GH initiation were again significant, and the model revealed an even greater imprinting effect (53%); the additional response in X^{mat} subjects relative to X^{pat} subjects was 0.64 SDS ($P = 0.031$; 95% CI, 0.06–1.21) or 5.22 cm ($P = 0.013$; 95% CI, 1.24–9.20). Identical models were examined in control subjects, using time of enrollment into the study as initiation. There were no statistically significant effects of baseline age, baseline height SDS, or parental origin of X^{intact} upon change in height SDS for control subjects.

Effect of the parental origin of the X^{intact} on SNHL

Fifty of the 54 studied subjects underwent hearing evaluation. Of these, 23 (46%) had SNHL. The prevalence of SNHL was significantly greater in the X^{pat} subjects, of whom 67% (12 of 18) were affected compared with the X^{mat} subjects

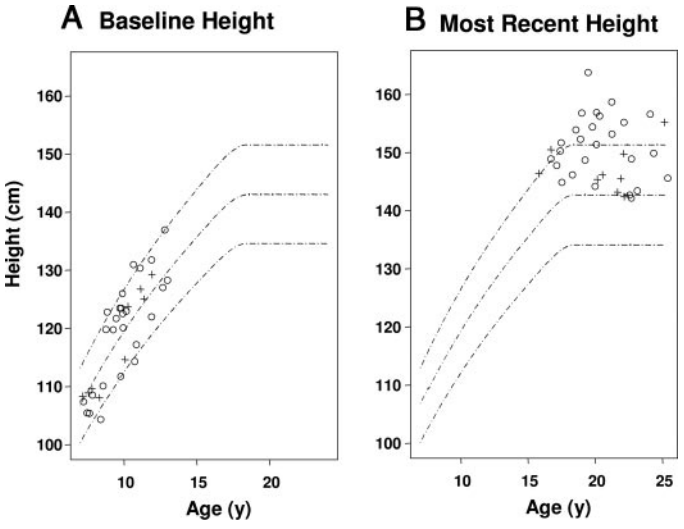

FIG. 3. Response to GH supplementation by parental origin of the intact X chromosome in 35 subjects with TS. At baseline (A), height of X^{mat} and X^{pat} subjects was comparable. At most recent height (B), 50% of the X^{mat} subjects had attained an adult height of at least the 90th percentile on the Turner-specific Lyon growth curve *vs.* only 33% of the X^{pat} subjects. ○, X^{mat} (n = 26); +, X^{pat} (n = 9).

of whom only 34% (11 of 32) were affected (Fisher's exact test, $P = 0.040$). No GH effect was detected. This suggests that X^{mat} subjects may express an X-linked imprinted gene that is important for normal sensorineural hearing function.

Discussion

This study provides evidence for an X-linked imprinting effect on GH response and on the occurrence of SNHL in girls with TS. Our findings reinforce the concept that X-linked imprinting is a significant mechanism of gene regulation in humans.

Whereas GH-treated subjects in this Canadian randomized controlled trial had a mean adult height gain of 7.3 cm relative to controls (11), our study adds the parental origin of the X^{intact} to the list of factors involved in the GH-Tx response, in the context of a standardized GH dose and pubertal induction regimen and after accounting for age and height at initiation of GH-Tx. Our results suggest that a maternally derived X chromosome may preferentially express a growth-promoting gene (or genes) that may influence GH efficacy. X-linked imprinting explained 36% (total group) to 53% (45,X group) of the adult height gain achieved with GH-Tx in X^{mat} subjects in a regression model that also accounted for age and height at initiation of GH-Tx. This is of clinical significance given that the average cost per year of GH-Tx is approximately $25,000 (Canadian). Additionally, as reported by others, we found a correlation between baseline height SDS of our subject population and midparental height. This observation is commonly used in clinical practice to assess whether a given growth channel (expressed as percentile on the Lyon growth curve (26)), corresponds to the patient's genetic potential (27). However, as we and Chu *et al.* (16) show, this correlation appears to be attributable primarily to the underlying correlation between maternal height and the presence of the intact X^{mat} in the majority of subjects with TS. It may reflect the effect of one or more growth-regulating genes expressed from the X^{mat} chromosome, although the presence of autosomal growth-regulating genes also contributes to spontaneous growth in TS.

Ogata and Matsuo (33) proposed that adult height in patients with sex chromosome aberrations may be defined by the dosage effect of pseudoautosomal genes. The discovery of *SHOX* led to the hypothesis that short stature in TS is caused, at least in part, by haploinsufficiency for this gene (5). *SHOX* is expressed exclusively in the developing distal limbs and in the first and second pharyngeal arches, where TS skeletal features are observed postnatally (34). To date, no data have been provided to suggest that *SHOX* is, or is not, imprinted in 46,XX individuals with preferential expression from the maternal allele, and given its location in the pseudoautosomal region, imprinting is unlikely.

Other data support our observations that an X^{mat} and an X^{pat} are not equivalent and may influence growth differently. A patient with a $45,X^{pat}/46,X^{pat}X^{pat}$ karyotype was reported as being shorter than would be expected despite the fact that greater than 90% of the cells contained two X chromosomes (35), suggesting that paternal isodisomy may have contributed to the phenotype. 46,X,i(Xq) individuals are also shorter than those with a 45,X karyotype (33, 36). We found that the X^{intact} was more frequently of paternal origin in subjects with a nonmosaic 46,X,i(Xq) karyotype; one possibility to explain the shorter stature in these subjects could be the absence of a growth-promoting gene from the short arm of the X preferentially expressed from the X^{mat} chromosome. It also raises the questions of whether there is a selective advantage to retaining an X^{mat}, either intact or rearranged, and whether the putative imprinted X-linked growth-determining gene(s) contribute to the height difference observed between genders.

The molecular basis for the predominance of X^{mat} among 45,X individuals is still not completely understood, although in part, it reflects the difficulty of detecting a low level of mosaicism as well as the nonviability of 45,Y zygotes. It is also likely that nonmosaic X monosomy arises preferentially from the loss of paternal sex chromosomes during spermatogenesis, meiotic I or II nondisjunction events (23), perhaps because of the weaker homology between X and Y chromosomes than between two X chromosomes. Increased proportions of XY and nullisomic sperm have, indeed, been observed in fathers of girls with TS compared with fathers of non-TS individuals (37). Paternal age does not appear to play a role because parental ages do not differ between X^{mat} and X^{pat} individuals with TS (23, 38–40). Hypotheses for X^{mat} predominance include problems at the pronuclear stage after sperm entry into the egg (41) as well as the precarious localization of the sex chromosome within the sperm head close to the acrosome, the site of gamete fusion (42). X-linked imprinting has also been suggested to play a role in intrauterine viability (43). However, a skewed ratio with predominance of X^{mat} has also been observed in aborted conceptuses, suggesting that imprinting is unlikely related to greater embryonic survival of X^{mat} conceptuses with TS (39, 40, 44). It is not clear, however, whether the presence of an intact X^{mat} would favor implantation, because preimplantation embryos with TS have not been studied.

To date, most studies have suggested that i(Xq) is equally likely to arise from a maternal or a paternal chromosomal error, because the X^{mat}:X^{pat} ratio is close to 1:1 when both mosaic [45,X/46,X,i(Xq)] and nonmosaic 46,X,i(Xq) groups are combined (28, 31, 38, 45, 46). To address the difference between our results and those of previous studies, we reviewed all nonmosaic 46,X,i(Xq) individuals reported in the literature and combined the data with our own (Table 3). In this analysis, the X^{mat}:X^{pat} ratio is 1:1.8 (deviation from expected 1:1 ratio, $P = 0.106$) (16, 22, 23, 28, 31, 38, 39, 45–52). However, isochromosomes are also structurally heterogeneous, not only in terms of the amount of Xp material present but also in terms of the number of centromeres. These abnormal chromosomes can be formed either by centromere misdivision [non-isodicentric or i(Xq)] or by sister/homolog chromatid exchange and reunion mechanisms [isodicentric or idic(Xq)] (53); the origin (oogenesis or spermatogenesis) and timing (meiosis I or II) of the cytogenetic error may differ. When we confine parental origin studies, including ours, to only the nonmosaic non-isodicentric 46,X,i(Xq) individuals, the preponderance of intact X^{pat} chromosomes increases (X^{mat}:X^{pat} = 1:3.4; n = 22), indicating that the isochromosome is of maternal origin in the majority of such patients. This ratio approaches a significant deviation from

TABLE 3. Summary of studies reporting parental origin of the intact X chromosome in patients with TS with an Xq isochromosome

Summary (Refs. 16, 22, 23, 28, 31, 38, 39, 45–52)	Overall		45,X/46,X,i(Xq)		46,X,i(Xq)	
	X^{mat}	X^{pat}	X^{mat}	X^{pat}	X^{mat}	X^{pat}
Without the present study	67 (14)	78 (24)	51 (10)	54 (12)	16 (4)	24 (12)
X^{mat}:X^{pat} ratio, including idic(Xq); n = 145	1:1.2		1:1.1		1:1.5	
X^{mat}:X^{pat} ratio, excluding idic(Xq); n = 38	1:1.7		1:1.2		1:3.0	
Including the present study	70 (17)	86 (31)	53 (12)	56 (14)	17 (5)	30 (17)
X^{mat}:X^{pat} ratio–including idic(Xq); n = 156	1:1.2		1:1.1		1:1.8	
X^{mat}:X^{pat} ratio, excluding idic(Xq); n = 48	1:1.8		1:1.2		1:3.4	

Numbers in parentheses include only non-isodicentric (Xq) cases.

a theoretical 1:1 ratio ($P = 0.058$) (Table 3). It is therefore important that parental origin studies look at homogeneous karyotypes as much as possible, particularly if we are to search for candidate imprinted genes.

SNHL, with or without an accompanying conductive hearing impairment, affects at least half of young women with TS (54, 55). The general course of SNHL is characterized by a reduction of hearing at the mid-frequencies in late childhood or early adulthood, which progresses over time to high frequency loss, resulting at age 40 in hearing comparable to that of women aged 60 in the general population (2, 48). The etiology of SNHL may involve lesions of the neural structures from the cochlea to regions of the auditory cortex (56). It has been suggested that hearing loss is caused by an X-linked dosage effect, because hearing deteriorates more rapidly in subjects with complete monosomy for the Xp arm, such as those with 45,*X* and 46,X,i(Xq) karyotypes, compared with those with smaller X deletions or with mosaicism including a normal 46,XX cell line (57). Our findings of a putative X-linked imprinting effect on SNHL suggest that a gene (or genes) expressed from the X^{mat} may prevent the gradual decline in hearing. Of note, patients with a 46,*X*,i(Xq) karyotype have the highest incidence of SNHL (54), perhaps because of the preponderance of intact X^{pat} in these individuals.

Other precedents for imprinting effects in the central nervous system exist in addition to data on cognitive function reported by Skuse *et al.* (17). Functional imaging studies have implicated abnormal patterns of cerebral activation in parietal and occipital regions in subjects with TS *vs.* controls (58). Brown *et al.* (59) showed a trend toward regional differences in brain volumes between X^{mat} and X^{pat} 45,*X* subjects; 45,X^{mat} subjects had larger volumes of the right and left superior temporal gyri, brain regions involved in language and hearing.

The inheritance asymmetry of the X chromosome between the sexes predisposes mammalian X-linked genes to have sex-specific expression controlled by imprinting. In humans, it was recently shown that 20% of X-linked genes are expressed from only some inactive X chromosomes derived from females with nonrandom X inactivation (60). This suggests a nonuniform behavior of gene expression that could be related to imprinting phenomena. We hypothesize that it will be these genes that will prove to be the most interesting candidates for parent-of-origin effects on X-linked gene expression.

Naumova *et al.* (61) have identified an imprinted locus at Xp11.4, a region of transmission-ratio distortion in human male offspring, which has been implicated in the viability of male embryos. To date, no candidate gene has been isolated, although there are several genes in this region that sometimes escape inactivation (60). Additional X-linked imprinted genes have been described in mice and sheep (62–67), although X chromosome human homologs have not been fully investigated.

Candidate imprinted regions on the X chromosome of particular interest to both the growth and SNHL phenotypes are located on the short arm, and this region is also rich in genes showing variable expression between individual inactivated X chromosomes (60). A non-pseudoautosomal stature-determining critical region has been mapped between Xp22.1 and Xp11.2 (68) based on a series of patients with partial deletions of Xp. Studies of families with nonsyndromic SNHL have uncovered two loci on Xp (*DFN6*, between Xp22.2 and Xp22.11, and *DFN4*, at Xp21.2) (56). Two additional SNHL loci also exist on Xq, but the hearing deficit phenotype in these families is different from that seen in TS. None of these loci have been explored for imprinting.

In conclusion, our findings suggest significant X-chromosomal imprinting effects on growth and SNHL in TS. Additional studies comparing expression of X-linked growth genes are needed to determine whether expression differs according to parent of origin of the X chromosome.

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